Role of Basophils in Aspirin Exacerbated Respiratory Disease Pathogenesis

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ABSTRACT:

Rationale: AERD is characterized by an enhanced type 2 inflammatory phenotype. Basophils are potent type 2 effector cells, but their involvement in AERD pathophysiology remains unclear. With this study, we sought to characterize the systemic and local basophil responses in patients with AERD compared to patients with CRSwNP.

Methods: Sinonasal tissues including inferior turbinate and/or nasal polyps (NPs) and peripheral blood were collected from controls, patients with AERD, and patients with CRSwNP. Expression of cell surface (CD45, FcεRI, CD203c), activation (CD63), and intracellular (2D7) markers associated with basophils was characterized using flow cytometry. Clinical data including Lund-Mackay scores and pulmonary function were obtained.

Results: The mean number of basophils ($CD45^+CD203c^+FcεRI^+CD117^-$) detected in AERD NPs (147±28 cells/mg tissue) was significantly elevated compared with that detected in CRSwNP NPs (69±20 cells/mg tissue; p = .01). The number of circulating basophils was significantly elevated in patients with AERD (p =.04). Basophils in NPs had significantly higher CD203c and CD63 mean fluorescence intensity compared with blood in both conditions (p < .01). Basophils from AERD NPs had lower expression of the granule content marker 2D7 compared with those from matched blood (p < .01) or NPs of patients with CRSwNP (p =.06), suggesting ongoing degranulation. Basophil 2D7 mean fluorescence intensity significantly correlated with pulmonary function (r = 0.62; p = .02) and inversely correlated with sinonasal inflammation (r = 20.56; p = .004).

Conclusions: Increased basophil numbers and extent of ongoing degranulation in NPs of patients with AERD compared with patients with CRSwNP may contribute to the exaggerated disease pathogenesis and severity unique to AERD.

INTRODUCTION:

Aspirin Exacerbated Respiratory Disease (AERD) is defined as a clinical triad consisting of chronic rhinosinusitis with nasal polyps (CRSwNP), asthma, and sensitivity to inhibitors of the cyclooxygenase-1 enzyme. This disease is associated with significant morbidity, large socioeconomic burden, and negative impact on quality of life. AERD patients, on average, have more severe objective measures of sinus inflammation, undergo increased numbers of sinus surgeries, and are more likely to require chronic oral corticosteroids when compared to patients with CRSwNP or asthma alone.[1,2] The underlying cellular and molecular mechanisms contributing to the enhanced severity and unique phenotype of AERD are not fully understood.

In Western countries, nasal polyps (NPs) are predominantly characterized by type 2 inflammation, with elevated levels of IL-5 and IL-13 in NPs when compared with healthy controls.[3,4] Cells commonly associated with type 2 inflammation, including type 2 $CD4^+$ T cells, lymphoid cells, mast cells, and eosinophils are elevated in NPs in the West.[5,6] Basophils are potent granulocytes that have long been associated with type 2 inflammation, but despite evidence that they are important contributors to respiratory diseases, there are few studies evaluating basophils in NPs. In a previous study, our group used immunohistochemistry to detect $2D7^+$ basophils and found elevations of these cells in CRSwNP tissue, but paradoxically not in AERD.[7] Limitations of that study prevented us from quantifying these cells rigorously by assessing the level of basophil activation and it did not relate basophils or basophil activation to markers of disease severity. With this study we significantly advanced our previous work on basophils by using a flow cytometry–based approach to characterize, quantify, and compare basophils in NPs of patients with AERD and CRSwNP. We found that basophils were significantly elevated and activated in NPs of patients with CRSwNP and in NPs of patients with AERD. We also found that basophil degranulation, measured by a decrease in 2D7 expression, correlated with enhanced clinical disease severity.

METHODS:

Subjects: Patients with CRSwNP or AERD were recruited from the Division of Allergy-Immunology and the Department of Otolaryngology clinics within Northwestern Medicine and all signed informed consent. Subjects met the criteria for CRS as previously defined[7] and the presence of sinusitis and bilateral NP was confirmed by endoscopy and/or sinus computed tomography imaging. All AERD patients had physician-diagnosed asthma, CRSwNP, and documentation of at least 1 respiratory reaction to a non-steroidal anti-inflammatory drug. NP tissue was obtained during routine endoscopic sinus surgery.

Cell Collection and Flow Cytometric Analysis: NP obtained during surgery were processed as previously described[8]. Cells were first treated with Aqua LIVE/DEAD fixable dead cell staining reagent as a live/dead discriminator. Cells were then incubated with antibodies including CD45, CD203c, CD117, FcεRI, CD63 and Siglec-8. For select samples, cells were then fixed and permeabilized for 2D7 intracellular staining. A LSRII flow cytometer was used and all subsequent analysis and compensation were performed with FlowJo. Each experiment contained the proper single-stained control beads and fluorescence minus one negative controls.

Statistical Analyses: Data were analyzed using a chi-square test, Mann-Whitney test, 1-way ANOVA Kruskal-Wallis test with Dunn's test for multiple comparisons, and Spearman rank as appropriate. A p ≤ .05 was considered significant and calculations were performed using Graphpad Software v6.0.

The Institutional Review Board of Northwestern University Feinberg School of Medicine approved this study.

Figure 1. Gating strategies for eosinophils, mast cells and basophils.

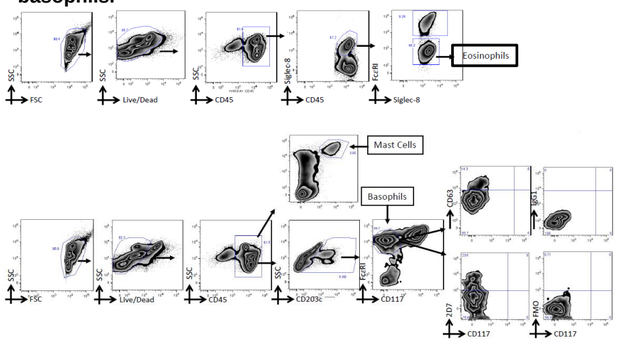

Figure 1. Representative gating strategy identifying eosinophils, mast cells, and basophils in NPs in a patient with AERD. Eosinophils were identified as Live/Dead$^-$CD45$^+$Siglec-8$^+$FcεRI$^-$ cells. Mast cells were identified as Live/Dead$^-$ CD45$^+$CD117$^+$FcεRI$^+$ cells, whereas basophils were identified as Live/Dead$^-$ CD45$^+$CD203c$^+$CD117$^-$FcεRI$^+$ cells. *FSC*, Forward scatter; *SSC*, side scatter.

Figure 2. Basophils were elevated in NPs of patients with AERD and correlated with the number of mast cells and eosinophils.

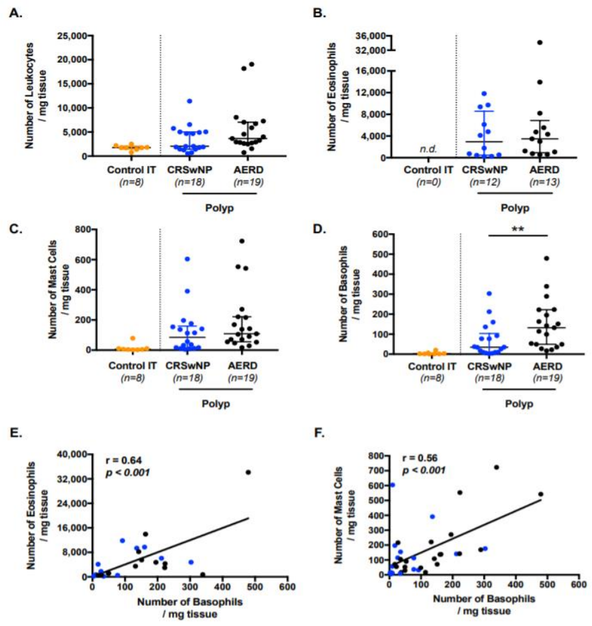

Figure 2. Flow cytometry was used to quantify the number of CD45$^+$ leukocytes (**A**), CD45$^+$Siglec-8$^+$FcεRI$^-$ eosinophils (**B**), CD45$^+$CD117$^+$FcεRI$^+$ mast cells (**C**), and CD45$^+$CD203c$^+$FcεRI$^+$CD117$^-$ basophils (**D**) in NPs of patients with CRSwNP or AERD. For comparison, the number of total leukocytes (**A**) and basophils (**D**) was quantified in inferior turbinate tissue of healthy controls. The number of tissue basophils strongly correlated with the number of tissue eosinophils (**E**) and tissue mast cells (**F**) in NPs. Statistical significance was determined by Mann-Whitney U test comparing AERD and CRSwNP (**A-D**) or Spearman rank correlation (**E** and **F**). $**p$ < .01. IT, Inferior turbinate; n.d., not determined.

Figure 3. Basophils were elevated in the circulation of patients with AERD compared to those with CRSwNP and correlated with the number of eosinophils in peripheral blood.

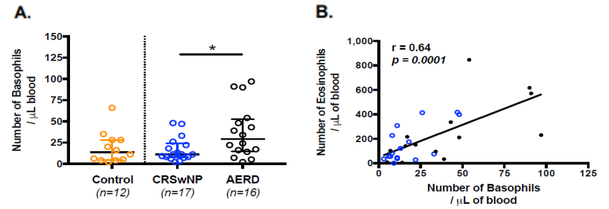

Figure 3. Flow cytometry was used to quantify the number of CD45$^+$CD203c$^+$FcεRI$^+$CD117$^-$ basophils (**A**) in the peripheral blood of patients with CRSwNP or AERD or healthy controls. The number of peripheral blood basophils strongly correlated with the number of circulating eosinophils (**B**) in patients with CRSwNP and AERD combined. Statistical significance was determined by Mann-Whitney U test comparing AERD and CRSwNP (**A**) or Spearman rank correlation (**B**). $*p$< .05.

Figure 4. Correlation between basophils in NPs and blood was driven primarily by patients with AERD.

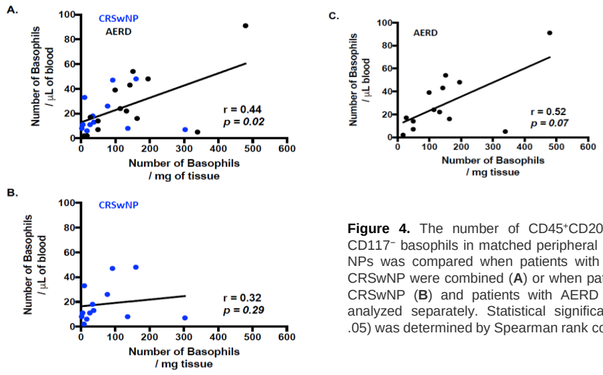

Figure 4. The number of CD45$^+$CD203c$^+$FcεRI$^+$ CD117$^-$ basophils in matched peripheral blood and NPs was compared when patients with AERD or CRSwNP were combined (**A**) or when patients with CRSwNP (**B**) and patients with AERD (**C**) were analyzed separately. Statistical significance (p < .05) was determined by Spearman rank correlation.

Figure 5. Basophils were more activated in NPs compared with peripheral blood but with more CD63^{hi} basophils in NPs of AERD than CRSwNP.

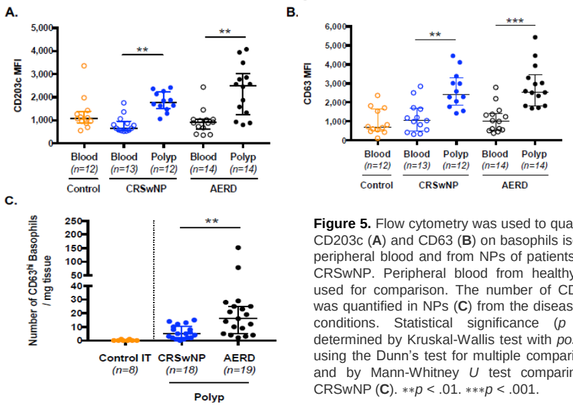

Figure 5. Flow cytometry was used to quantify the MFI of CD203c (**A**) and CD63 (**B**) on basophils isolated from the peripheral blood and from NPs of patients with AERD or CRSwNP. Peripheral blood from healthy controls was used for comparison. The number of CD63^{hi} basophils was quantified in NPs (**C**) from the diseased and healthy conditions. Statistical significance (p < .05) was determined by Kruskal-Wallis test with *post hoc* analysis using the Dunn's test for multiple comparison (**A** and **B**) and by Mann-Whitney *U* test comparing AERD and CRSwNP (**C**). $**p$ < .01. $***p$ < .001.

Figure 6. Basophil degranulation was enhanced in NPs of patients with AERD compared to those with CRSwNP.

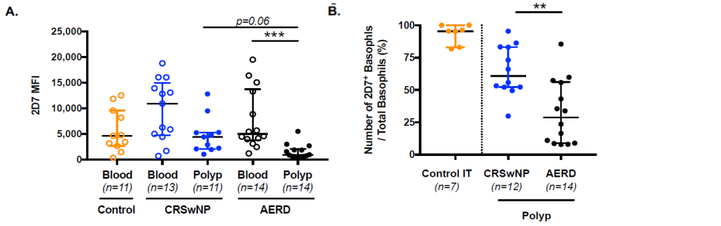

Figure 6. Flow cytometry was used to quantify the MFI of intracellular 2D7 expression (**A**) within basophils isolated from NPs of patients with AERD or CRSwNP The percentage of basophils expressing 2D7 in NPs (**B**) was also determined. Statistical significance (p < .05) was determined by Kruskal-Wallis test with *post hoc* analysis using the Dunn's test for multiple comparison (**A**) and by Mann-Whitney *U* test comparing AERD and CRSwNP (**B**). $*p$ < .05. $***p$ < .001.

Figure 7. Markers of basophil activation (CD63) and degranulation (2D7) correlated with sinonasal and pulmonary disease severity.

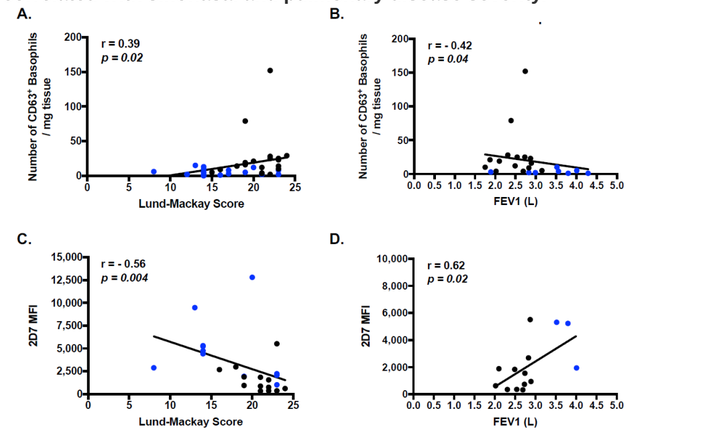

Figure 7. The number of CD63^{hi} basophils significantly correlated with the LM score (**A**) and inversely correlated with FEV_1 (**B**) in patients with either CRSwNP or AERD. In addition, 2D7 MFI inversely correlated with the LM score (**C**) and positively correlated with FEV_1 (**D**) in both CRSwNP and AERD. Dot plots illustrate individual data points, with blue representing a patient with CRSwNP and black representing a patient with AERD. Statistical significance (p < .05) was determined by Spearman rank correlation.

SUMMARY:

- Basophils were elevated in NPs and peripheral blood of patients with AERD compared with patients with CRSwNP
- NP basophils in AERD expressed less 2D7 suggesting enhanced degranulation compared to NP basophils in CRSwNP
- Markers of basophils activation and degranulation strongly correlated with clinical markers of enhanced disease severity

CONCLUSION:

Basophils may contribute to the exaggerated pathogenesis and enhanced clinical disease severity observed in patients with AERD.

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